repeated measurement. We have used this platform to measure the interactions of aminated and carboxylated polystyrene nanoparticles and a range metal oxide nanoparticle species with lipid bilayers in a wide variety of experimental conditions, including nanoparticle concentration, bilayer composition, bilayer charge, presence of serum protein, solution ionic strength, and pH. The array format permitted several thousand bilayers to be measured in total with sufficient redundancy to give statistical significance to measured results. Detailed analysis of the electrical measurements shows pore formation that is dependent on electric field, ionic strength, and nanoparticle species.

1. ''Lipid bilayer arrays cyclically formed and measured,'' Bin Lu, Gayane Kocharyan and Jacob J. Schmidt, Biotechnology Journal 9, 446-451 (2014)

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Sticky Patches on Lipid Nanoparticles Generate Binding Geometries that Enable Effective Targeting of Otherwise Untargetable Cancers

Michelle Sempkowski[1], Yannis Kevrekidis[2], **Stavroula Sofou**[1].
[1]Rutgers University, Piscataway, NJ, USA, [2]Princeton University, Princeton, NJ, USA.

The majority of breast cancer patients (70%) have tumors designated as 'HER2-negative' (<1+ HER2-expression evaluated by immunohistochemistry or < 200,000 HER2-copies per cell). For these patients there are no targeted therapeutic options utilizing the HER2 receptor. The ability of conventionally targeted nanoparticles for specific targeting stops to hold on cancer cells expressing less than 200,000 copies of HER2 per cell or less than two receptors per nanoparticle's projected area (for particles of 100 nm in-diameter). This geometry corresponds to the limit of multivalent interactions (avidity) loosely defined as multiple contacts between neighboring same-cell receptors with ligands from a single nanoparticle. An alternative therapeutic approach is needed, therefore, to enable selective targeting and effective killing of cancer cells with low or too low HER2 expression.

Towards this goal we designed targeted lipid nanoparticles (vesicles) that contain HER2-targeting short peptides densely conjugated (for high local multivalency) within sticky patches. Sticky patches are phase-separated raft-like lipid-domains of high local multivalency which is induced by preferential partitioning of peptide-functionalized lipids. To enable selectivity in binding, sticky patches are exclusively triggered to form in mildly acidic environments matching the tumor interstitium. Lipid phase-separation with lowering pH is a result of the interplay of decreasing (pH-tunable) electrostatic repulsion and attractive hydrogen bonding among the domain-forming lipids.

We show that lipid nanoparticles with sticky patches selectively associate with and kill HER2-negative and triple negative breast cancer cells (MCF-7 and MDA-MB-231, respectively, with 60,000 and 90,000 HER2-copies per cell) while do not affect cardiomyocytes and breast normal cells. Systematic studies of association, dissociation and internalization rates of nanoparticles by cells will be presented, and a mechanistic mathematical model will be discussed with the aim to explain the observed high avidity.

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Controlled Activation of Protein Rotational Dynamics using Smart Hydrogel Tethering

Yijia Xiong[1], Brenda M. Beech[2], Curt B. Boschek[2], Cheryl L. Baird[2], Diana J. Bigelow[2], Kathleen McAteer[3], **Thomas C. Squier**[1].
[1]Basic Medical Sciences, Western University of Health Sciences, Lebanon, OR, USA, [2]Biological Sciences, Pacific Northwest National Laboratory, Richland, WA, USA, [3]Biological Sciences, Washington State Universtiy Tri-Cities, Richland, WA, USA.

Stimulus-responsive hydrogel materials that stabilize and control protein dynamics have the potential to enable a range of applications that take advantage of the inherent specificity and catalytic efficiencies of proteins. Here we describe the modular construction of a hydrogel using an engineered calmodulin (CaM) within a polyethylene glycol (PEG) matrix that involves the reversible tethering of proteins through an engineered CaM-binding sequence. For these measurements, maltose binding protein (MBP) was isotopically labeled with [^{13}C] and [^{15}N], permitting dynamic structural measurements using TROSY-HSQC NMR spectroscopy. Protein dynamics are suppressed upon initial formation of hydrogels, with concomitant increases in protein stability. Relaxation of the hydrogel matrix following transient heating results in enhanced protein dynamics and resolution of substrate-induced large-amplitude domain rearrangements.

Our results demonstrate an ability to take advantage of the conformational sensitivities of hydrogel materials to activate protein dynamics upon transient temperature increases. Such an approach permits storage of proteins in an immobilized state prior to their activation, and contributes to important applications that can take advantage of the specificity of proteins for a range of sensing and chemical transformation applications. For example, single chain antibodies are shown to be dramatically stabilized against denaturation by urea, enabling their long term use for sensing applications. These smart materials possess optimized mass transfer properties (due to their high water content) and provide important avenues to detect ligands so as to link binding to material responses (e.g., proteolysis or other types of chemical transformation using the catalytic specificities of enzymes).

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Immobilization of Proteins on Chemically Modified Germanium Investigated by ATR-FTIR

Jonas Schartner, Konstantin Gavriljuk, Andreas Nabers, Klaus Gerwert, Carsten Kötting.
Biophysics, Biology & Biotechnology, Bochum, Germany.

The attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) allows a detailed analysis of surface attached molecules, including their secondary structure, reaction mechanism, orientation and interaction with small molecules or proteins.[1] The aim of our study is the development of a universal immobilization technique on germanium for all kinds of proteins. We recently showed the specific immobilization of N-Ras and Photosystem I on a silane modified germanium surface.[1]

We now present a new approach employing thiol chemistry on germanium.[2,3] On one hand germanium crystals provide a great signal-to-noise ratio in ATR-FTIR. On the other hand protein immobilization via thiol chemistry is well-established because it is standard for modifications of gold surfaces e.g. in surface plasmon resonance. Here we combine the best of both worlds and report on germanium surface functionalization with different thiols which allowed for specific immobilization of histidine-tagged proteins with over 99% specific binding. The great advantage of using thiols in comparison with silanes is that a huge variety of thiols with functional groups for many kinds of protein immobilizations are readily available and the higher stability. Nativity of protein folding was confirmed by secondary structure analysis. Stimulus induced difference spectra were obtained for immobilized Channelrhodopsin 2, the small GTPase N-Ras and the phosphocholine-transferase AnkX, which demonstrated protein function at the atomic level.[4] Protein activity was observed for Channelrhodopsin 2 for over several days.[4]

1: **Schartner J**. *et al.*, *J. Am. Chem. Soc*., 2013, 135, 4079-4087
2: **Hanrath, T. & Korgel**, B. A., *J. Am. Chem. Soc*., 2004, 126, 15466-15472
3: **Han, S**. *et al*. *J. Am. Chem. Soc*., 2001, 123, 2422-2425
4: **Schartner J**., *et al., ChemBioChem*, 2014, *accepted*

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Use of Short Amyloidogenic Peptides in Protein-Ligand Detection Systems

Gabriela M. Guerra, Sónia Gonçalves, Nuno C. Santos, **Ivo C. Martins**.
Biomembranes Unit, Institute of Molecular Medicine (IMM), Lisbon, Portugal.

Amyloid fibers, often associated with many human degenerative diseases (such as Alzheimer's and Parkinson's disease), may also have physiological roles, having even been suggested as potential novel biomaterials [1-2]. Since it is now clear that the amyloid fibers are much less toxic than their precursor aggregates [3], the interest for amyloidogenic species in nanosensing and protein-ligand detection sytsems increased dramatically [2]. Amyloid fibers in general share a common β-sheet rich architecture that is behind their exceptional stability, mechanical strength and resistance to degradation, which in nanotechnology makes them excellent nanomaterials candidates [1]. The potential to form amyloids (and other protein/peptide aggregates) can be predicted from the peptide amino acids sequence [1, 2]. Here, we used different amyloid peptide sequences to evaluate, by AFM, circular dichroism and FTIR spectroscopic approaches under different conditions, which type of amyloid species would be formed (namely amyloid oligomers, protofibrils or fibrils) at different times of incubation (24 hours, 72 hours and 2 weeks). AFM, CD and FTIR data taken together indicate that amyloid-based nanotechnology approaches may be successfully employed.

References (* stands for the presenting author own work)
[1] Cherny & Gazit, 2008, Angew Chem Int Ed Engl, 47:4062
[2] Hauser et al., 2014. Chem Soc Rev, 43:5326*
[3] Martins et al., 2008. EMBO J, 27:224*
[4] Maurer-Stroh et al., 2010, Nat Methods, 7:237*